Standard Operating Procedure for Total and Total Dissolved Phosphorous (Lachat Method 10-115-01-1-F for QuikChem FIA+8000)

LG204

Revision 07, March 2005

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1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of Total and Total Dissolved Phosphorous in lake water.

1.2 The approximate working range is 1.0 to 50.0 µg/L. The method detection limit is 1.0 µg/L.

2.0 SUMMARY

2.1 Samples are digested in the presence of sulfuric acid and persulfate to convert or "hydrolyze" polyphosphate and organic phosphorous to orthophosphate.

2.2 The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form 12 molybdophosphoric acid. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3.0 SAMPLE HANDLING AND PRESERVATION

3.1 Samples are collected in new plastic containers.

3.2 Samples are preserved by addition of 1 mL of H_2SO_4 per liter of sample.

4.0 INTERFERENCES

4.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant. A silica concentration of 50 mg SiO_2/L is required to produce a 0.008 mg P/L positive error in orthophosphorous.

4.2 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed several times with reagent water. ***Special glassware (volumetric flasks, graduated cylinders, etc.) has been designated for TP/TDP/DRP ONLY use.***

4.3 High concentrations of ferric iron or arsenate ion can cause error due to competition with the complex for ascorbic acid. Such concentrations are highly unlikely in lake water.

5.0 APPARATUS

5.1 Digestion tubes: Borosilicate Glass 16 x 100 mm Culture Tubes with white polypropylene screw caps

5.2 Autoclave

5.3 Automatic pipets with disposable tips

5.4 Lachat QuikChem FIA+8000

5.4.1 Phosphate Manifold (Lachat Manifold # 30-115-01-1-B)

5.4.2 Printer

5.4.3 XYZ Sampler

6.0 REAGENTS AND STANDARDS

6.1 All reagents should be stored in the appropriate bottles and labeled with the following information:

Identity: (Ascorbic Acid)

Date of Preparation: (mm/dd/yy)

Expiration Date: (mm/dd/yy)

Initials of Preparer: (M.S.)

All standards should be stored in the appropriate bottles and labeled as above with the following also included:

Concentration: (100 mg P/L)

6.2 **Use reagent water for all solutions.**

6.3 **Sulfuric Acid Solution**. With continuous mixing, add 150 ml of concentrated sulfuric acid to 385 ml of Reagent water.**Very high temperatures are generated when sulfuric acid is mixed with water. Never add water to containers of sulfuric acid.This operation should be performed in a fume hood**.

6.4 **Ammonium Persulfate Concentrate:** In a 100 ml volumetric flask, dissolve 20.0 g ammonium persulfate [$(NH_4)_2S_2O_8$] in about 80 ml of regent water. Dilute to the mark.

6.5 Digestion Solution: Combine one part of 4.4 N H_2SO_4 (6.3) and one part of Ammonium Persulfate Concentrate (6.4)

6.6 Stock Ammonium Molybdate Solution: In a 1-L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6\ Mo_7O_{24}$] in approximately 800 mL reagent water. Dilute to the mark and invert to mix. Store in plastic and refrigerate.

6.7 Stock Antimony Potassium Tartrate Solution: In a 1-L volumetric flask, dissolve 3.0 g antimony potassium tartrate [$K(SbO)C_4H_4O_6 \bullet ½H_2O$] in approximately 800 mL of reagent water. Dilute to the mark and invert three times to mix. Store in a dark bottle and refrigerate.

6.8 Molybdate Color Reagent: In a 500 mL volumetric flask containing about 100 ml of reagent water, add 75.0 ml of Sulfuric Acid Solution (6.3). Swirl to mix. Add 36.0 ml of the **Stock Antimony Potassium Tartrate Solution** and 100.0 ml of the **Stock Ammonium Molybdate Solution.** Dilute to the mark. Degas with helium. Store in plastic at room temperature.

6.9 Ascorbic Acid Reducing Solution: In a 1-L volumetric flask dissolve 60.0 g ascorbic acid in about 700 mL of reagent water. Dilute to the mark and invert three times to mix. Degas with helium. Add 1.0 g dodecyl sulfate, sodium salt ($CH_3(CH_2)_{11}OSO_3Na$).

6.10 Sulfuric Acid Carrier Solution: In a 1-L volumetric flask containing about 900 mL of reagent water, add 95 mL of 4.4 N H_2SO_4 (6.3). Dilute to the mark. Invert to mix and degas with helium.

6.11 Sodium Hydroxide - EDTA Rinse: Dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylene diamine tetraacetic acid (Na_4EDTA) in 1 L of reagent water.

6.12 Hydrochloric Acid Rinse: Combine equal parts water and concentrated hydrochloric acid (HCl).

6.13 Preparation of Standards

6.13.1 Stock Calibration Standard (100 mg P/L): Dry a small amount of potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 2 hours. Cool to ambient temperature in a desiccator. In a 1-L volumetric flask, dissolve 0.4394 g of dried reagent in about 500 mL of reagent water. Add 3.2 mL of Sulfuric Acid Solution (6.3) and dilute to the mark. Store at 4°C.

6.13.2 Intermediate Calibration Standard (1.0 mg P/L): Transfer 10 mL of the Stock Calibration Standard (6.12.1) into a 1-L volumetric flask, containing about 500 mL of reagent water. Add 3.2 mL of Sulfuric Acid Solution (6.3) and dilute to the mark. Store at 4°C.
Alternatively Intermediate Calibration Standard (1.0 mg P/L) can be prepared from commercial 50 mg P/L Phosphate Standard. Transfer 20 ml of Commercial 50 mg P/L Phosphate Standard into a 1 L volumetric flask, containing about 500 ml of regent water. Add 3.2 ml of Sulfuric Acid Solution (6.3) and dilute to the mark. Store at 4°C.

6.13.3 Working Calibration Standards: Prepare standards over the range of analysis. For the working range of 0.0 - 50.0 μg/L, the following standards may be used:

mL Intermediate Calibration Standard (6.13.2) diluted to 1 L	Concentration μg P/L
0.0	**0.0**
2.5	**2.5**
5.0	**5.0**
7.5	**7.5**
10.0	**10.0**
25.0	**25.0**
50.0	**50.0**

NOTE: Use volumetric flasks. Preserve the working standards by addition of 3.2 mL of Sulfuric Acid Solution and store at 4 ℃.

6.13.4 Stock 100 mg P/L Control Standard: Dry a small amount of Adenosine-5-Monophosphoric Acid, Disodium salt, [($C_{10}H_{12}N_5O_7PNa_2 \bullet 2H_2O$), F.W. - 427.236 g/mole, Fluka] in an oven at 105°C for 2 hours. Allow to cool to room temperature in a desiccator. In a 1-L volumetric flask, dissolve 1.3793 g of the reagent in about 500 mL of reagent water. Add 3.2 mL of Sulferic Acid Solution (6.3) and dilute to the mark. Store at 4°C.
Alternatively Stock 100 mg P/L Control Standard can be prepared from Commercial 1000 mg P/L Organic Nutrients Standard. Transfer 100 ml of commercial 1000 mg P/L Organic Nutrients Standard into a 1 L volumetric flask, containing about 500 ml of regent water. Add 3.2 ml of Sulfuric Acid Solution (6.3) and dilute to the mark. Store at 4°C.

6.13.5 Intermediate Control Standard (1.0 mg P/L): Transfer 10.0 mL of the Stock Control Standard (6.12.4) into a 1-L volumetric flask, containing about 500 mL of reagent water. Add 1.0 mL of concentrated sulfuric acid and dilute to the mark. Store at 4°C.

6.13.6 Working Control Standards: The following concentrations are typical:

Working Control Standard ID	mL Intermediate Control Standard (6.13.5) diluted to 1 L	Concentration µg P/L
High Check Standard (CH)	**15.0**	**15.0**
Low Check Standard (CL)	**3.0**	**3.0**

NOTE: Preserve the control standards by addition of 3.2 mL of Sulfuric Acid Solution (6.3) Store at 4 ℃.

7.0 PROCEDURE

7.1 Digestion

7.1.1 DO NOT USE COMMERCIAL DETERGENTS. Soak digestion tubes in 1:1 HCl for 1 hour, rinse thoroughly with reagent water and allow to dry completely before use.

7.1.2 Using an automatic pipet with disposable tip, withdraw a 9.5 mL aliquot of sample. Discard this first portion. Withdraw another 9.5 mL aliquot and transfer to a digestion tube.

7.1.3 Add 0.5 mL of digestion solution (6.5).

7.1.4 Cap the tube tightly and place in metal digestion rack.

7.1.5 Prepare all samples, calibration standards, blanks, and control standards in the same manner.

7.1.6 Place the rack of tubes in an autoclave at 121°C for 30 minutes.

7.1.7 Allow the samples to cool to room temperature before analysis. Redigest any tubes that gain or lose volume.

7.2 Analysis

7.2.1 Allow at least 20 minutes for the heating block to warm up to 37°C.

7.2.2 Follow the Lachat Procedural SOP (Typical Daily Operation Section) for the remainder of the analysis.

7.2.3 At the end of a run, place all lines into the NaOH-EDTA solution (6.11). Pump this solution for approximately 5 minutes. Rinse lines with reagent water for another 5 - 10 minutes.

8.0 CALCULATIONS

8.1 The computer yields results directly in µg P/L**.**

9.0 QUALITY CONTROL

9.1 Refer to the Chapter 2 Introduction for definitions of quality control samples and information regarding quality control procedures, such as QC sample IDs and labeling.

9.2 The minimum acceptable correlation coefficient (r) is 0.995.

9.3 The following QC samples must be prepared and analyzed at the minimum frequency indicated.

QC Sample Type		Frequency	Acceptance Criteria
External	Field Reagent Blank (FRB)	One per basin[a]	0.0 ± 1.0 μg/L or less than one tenth associated field sample concentrations, whichever is greater
	Field Duplicate (FD1)	One per basin[a]	Difference ≤ 2 μg/L
	Lab Duplicate (LD1)	One per basin[a]	Difference ≤ 2 μg/L
Internal	Calibration	At the beginning of each batch	r ≤ 0.995
	High Check Standard (CH)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	15.0 ± 3.0 μg/L
	Low Check Standard (CL)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	3.0 ± 2.0 μg/L (interim)
	Laboratory Reagent Blank (LRB)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	0.0 ± 1.0 μg/L
	Method Detection Limit (MDL)	Once per year and each time a significant change is made to the SOP	1 μg/L

[a] **A field duplicate, lab duplicate, and field reagent blank are collected with each group of 3, 4, or 5 stations depending on the lake. A Random Number Generator (RNG) is used to determine the stations and depths of these QC samples. Where basins are well defined, at least one of each is collected from each basin.**

9.4 Assessment

9.4.1 The analyst must compare analytical results to the acceptance criteria listed in Section 9.3 to identify QC failures. If the results are outside the acceptance criteria, the analyst should first review their calculations for errors and if none are identified, they must follow the corrective action procedures listed in Section 9.5.

9.5 Corrective Actions

9.5.1 Corrective action procedures will often be handled at the bench level by the analyst, who reviews the preparation or extraction procedure for possible errors, checks the instrument calibration, spike and calibration mixes, instrument sensitivity, and any other potential sources of error. If failure occurs and an error is identified, the analyst should re-run quality control and RFS samples in the entire analytical batch to confirm the results. Because external QC samples are collected and prepared during the survey and provided to the contractor or grantee laboratory, a single rerun to confirm results is sufficient when all other QC samples are within acceptance criteria. For analyses conducted onboard, if the problem persists or cannot be identified, the matter must be referred to the Chief Scientist for further investigation. Depending upon the Chief Scientist's evaluation, the analyst may or may not be required to prepare and re-run the samples again. Additionally, if the results are significantly different than the expected concentrations based on historical data or related samples, then the analyst may split the RFS sample in the laboratory and analyze the splits. Once a decision is made, full documentation of the corrective action procedures and assessment of the final result must be filed with the WQS QM Technical Lead (Marvin Palmer) or the GLNPO QM. For analyses conducted at contract or grantee laboratories, this information can be included with submitted data. When contractor or grantee laboratories have a question regarding acceptable corrective actions, they should contact the Biology Technical Lead or Limnology Technical Lead as appropriate for instruction at a time when corrective action can still be taken.

9.6 Data Reporting/Recording

9.6.1 When corrective actions are not feasible or do not resolve QC failure, the analyst is responsible for identifying all failed QC samples and RFS samples. If analyses are being conducted onboard, the analyst should document the QC information on the hard-copy Field Information Recording Forms (Appendix H). If analyses are being conducted by contract or grantee laboratories, the analyst should document all QC information with the submitted data.

10.0 WASTE DISPOSAL

10.1 Effluent from this channel as well as the sample effluent is acidic. It should be disposed of in a yellow labeled waste container.

11.0 PREVENTATIVE MAINTENANCE

11.1 Required maintenance is described in the Lachat Procedural SOP.

12.0 TROUBLESHOOTING

12.1 If the baseline drifts and cleaning the system in the prescribed manner does not help, the heating coil tubing may need to be changed.

12.2 If negative peaks are observed in some or all of the samples or standards, it is probably due to matrix difference between the carrier and the samples. Check to be sure the carrier was made up properly and that the sulfuric acid addition to the digestate was not unintentionally omitted. Re-digest those samples that exhibited the negative peaks.

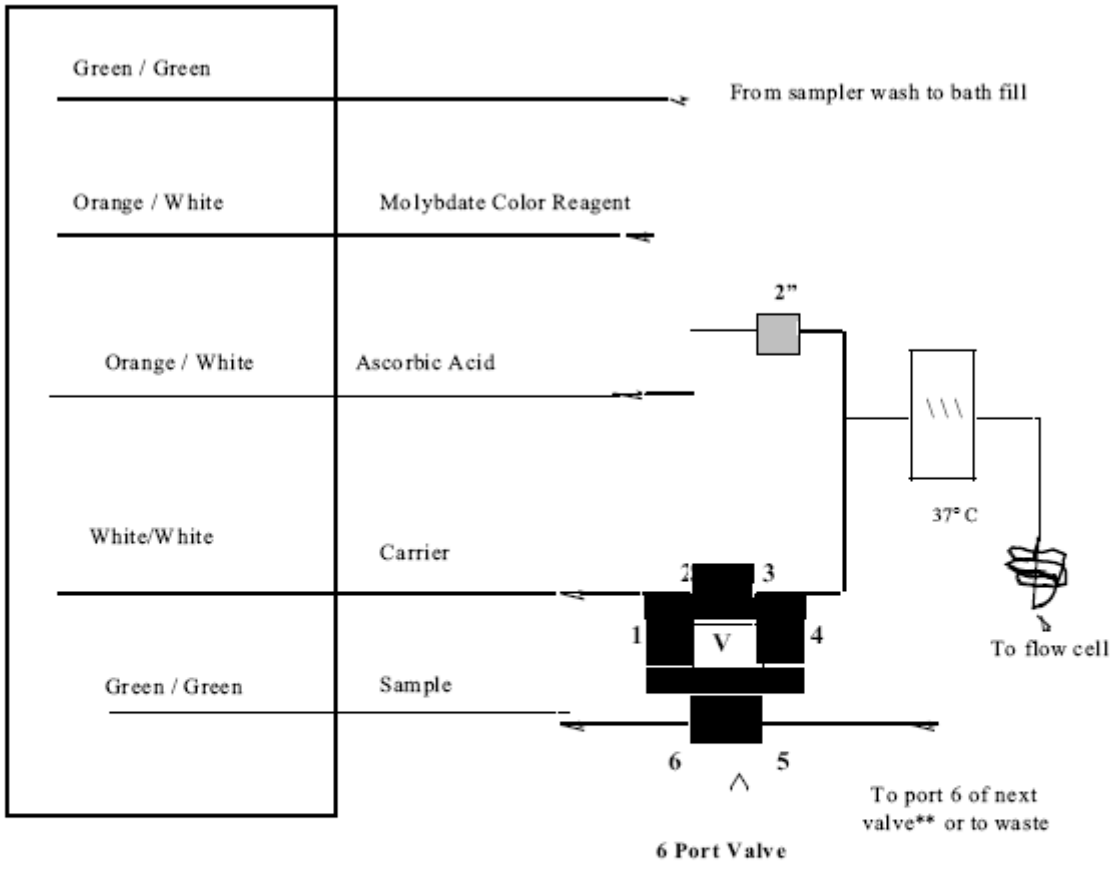
12.3 An unusually noisy baseline may be due to insufficient purging of air from the reagents. Tiny bubbles tend to develop in the heated tubing and may become trapped in the flow cell causing baseline problems. Prepare fresh Ascorbic Acid reagent (6.9) and thoroughly degas all other reagents.

13.0 REFERENCES

13.1 Lachat Instruments, Method Number 10-115-01-1-F, Determination of Total Phosphorus By Flow Injection Analysis Colorimetry (Acid Persulfate Digestion Method), Revision Date 13 August 1998.

13.2 Lachat QuikChem 8000 Automated Ion Analyzer, Omnion FIA Software and Hardware Installation and Operation Manual, Zellweger Analytics, 30 April 1998 Revision.

14.0 TOTAL AND TOTAL DISSOLVED PHOSPHOROUS ANALYTICAL MANIFOLD



Legend

- 2.0" Mixing coil (there is 135 cm of tubing on the 2.0" coil support)

- The box shows 175 cm of tubing warped around the block heater.

Comments

1. Filter used is 880 nm.
2. Sample loop length is 300 cm.
3. All manifold tubing is 052 nm (0.022") ID. This related to a flow of 5.2 L/cm.
4. 3 m back pressure loop 0.52 mm (0.22") ID.
5. The carrier is Reagent 6.10.

** If more than one channel is being used.